Published by Elsevier Inc.

ISSN 0735-1097/04/$30.00
doi:10.1016/j.jacc.2004.03.023

EDITORIAL COMMENT

Soluble CD40 Ligand and Platelets: Self-Perpetuating Pathogenic Loop in Thrombosis and Inflammation?*

Pål Aukrust, MD, PHD,†‡
Jan Kristian Damås, MD, PHD,†
Nils Olav Solum, PHD†
Oslo, Norway

Platelet adhesion and aggregation at the site of plaque rupture is a crucial event in thrombus formation and the development of acute coronary syndrome (ACS). However, recent studies suggest that platelets also may trigger ACS through other mechanisms such as the stimulation of an inflammatory response. Thus, several studies suggest a role for platelets as inflammatory cells (1,2). First, platelets release a wide range of inflammatory mediators from intracellular stores, such as several chemokines. Second, platelets not only express and release inflammatory mediators but may upon activation also induce the expression of such substances in other cells (e.g., monocytes/macrophages and granulocytes). Finally, platelets may themselves respond to inflammatory mediators. In fact, platelets recently have been found to express several chemokine receptors that upon stimulation enhance platelet activation. Hence, during activation platelets may not only promote thrombus formation but also seem to release and express inflammatory mediators, induce an inflammatory response within leukocytes and endothelial cells, and respond with activation to several of the inflammatory mediators produced by these cells.

See page 2319

Recently, much attention has been focused on the role of platelet-derived CD40 ligand (CD40L) in this inflammatory loop between platelets and other cells (2,3). Platelet-derived CD40 ligand, a transmembrane protein structurally related to tumor necrosis factor-alpha, originally was identified on $CD4^+$ T cells but has recently been found also on mast cells, basophils, eosinophils, and activated platelets (2,3). Soluble CD40L (sCD40L) is found to be elevated in coronary disease, particularly in patients with ACS (4), and has been associated with increased cardiovascular risk in apparently healthy women (5). Several lines of evidence suggest that these findings not only represent epiphenomena but also may reflect important pathogenic processes in these patients. Both membrane-bound and sCD40L may interact with CD40—which is constitutively expressed on a wide range of cells such as macrophages, endothelial cells, vascular smooth muscle cells, and platelets—resulting in various inflammatory responses (2,3,6). Thus, the in vitro stimulation of CD40 signaling in atheroma-derived cells results in the production of cytokines, tissue factor, matrix metalloproteinases (MMPs), and adhesion molecules (2,3,6). In vivo, an important role for the CD40L-CD40 interaction in atherogenesis was demonstrated using mice that were deficient in CD40L and apolipoprotein E (apoE) showing a dramatic decrease in plaque area in these mice compared with normal apoE-deficient animals (7). The possible plaque-stabilizing effect of CD40L neutralization was further demonstrated in another study where the administration of anti-CD40L antibody to apoE-deficient mice induced a stable plaque phenotype (8).

Recently, the list of biological activities of CD40L was extended from regulation of immune responses to induction of platelet activation and thrombus formation. Thus, André et al. (9) showed that CD40L can stabilize arterial thrombi by an intergrin-dependent mechanism. It is unclear whether both the transmembrane and the soluble forms of CD40L are active in promoting platelet activation, but recombinant sCD40L alone was shown to enhance integrin-mediated platelet aggregation at high shear rates, restoring normal thrombosis in $CD40L^{-/-}$ mice (9). These authors have more recently suggested that sCD40L is a ligand for glycoprotein (GP) IIb/IIIa, inducing platelet stimulation as evidenced by the generation of platelet microparticles (10). On the other hand, others have suggested that sCD40L may activate platelets through CD40 ligation, inducing release from both alpha- and dense-granule (11). Whatever mechanism, these studies suggest that CD40L may be involved in thrombus formation and platelet activation, leading to further increase in sCD40L release, representing a self-perpetuating pathogenic loop in this process.

It is estimated that >95% of the circulating sCD40L is derived from platelets. Therapeutic strategies aiming to block the interaction between sCD40L and CD40 should therefore focus on the possibility of blocking the release of sCD40L from these cells. It was originally suggested that sCD40L, upon platelet activation, was released from alpha-granule (4), but in contrast to the rapid release from these granules (within minutes), the release of sCD40L is much slower, with a maximum between 45 and 100 min (12). The prevailing idea today is, therefore, that CD40L is surface-exposed in a fast process and that sCD40L is then released from this membrane-bound CD40L as a degradation product in a slow proteolytic process. However, at present ambiguity exists regarding the exact mechanisms for sCD40L generation. In this issue of the *Journal*, Furman et al. (13) extend our knowledge of the regulation of sCD40L release from platelets. First, they show that GP IIb/IIIa

*Editorials published in the *Journal of the American College of Cardiology* reflect the views of the authors and do not necessarily represent the views of *JACC* or the American College of Cardiology.

From the †Research Institute for Internal Medicine; and ‡Clinical Immunology and Infectious Diseases, Rikshospitalet, Oslo, Norway.

activation is involved in the release of sCD40L, showing a blocking effect of GP IIb/IIIa antagonist, as also demonstrated by others (14), and by showing that patients with Glanzmann thrombasthenia, an inherited deficiency of GP IIb/IIIa on platelets, have a markedly decreased release of sCD40L upon platelet activation. Second, the authors also show that although GP IIb/IIIa blocking or deficiency reduced sCD40L release, it did not affect the activation-dependent translocation of CD40L to the platelet surface, which is in line with the idea that the up-regulation of the membrane-bound CD40L and release of sCD40L in platelets are regulated differently.

Glycoprotein IIb/IIIa antagonists block the binding of soluble fibrinogen to the GP IIb/IIIa receptor on the platelet surface, thereby inhibiting platelet aggregation. The effectiveness of intravenous GP IIb/IIIa antagonists in preventing ischemic complications after percutaneous coronary intervention has been well documented. Interestingly, some trials suggest that the clinical benefit extends well past the time of short-term administration of GP IIb/IIIa antagonists (15). Herein, Furman et al. (13) show that GP IIb/IIIa antagonists are able to inhibit the release of sCD40L from activated platelets, and others have previously shown that these antagonists may reduce the platelet CD40L-mediated activation of endothelial cells (16). These observations may suggest that long-term benefit of these antagonists could be due not only to the inhibition of the short-term ischemic complications but also to the inhibition of the autocrine function of sCD40L, interrupting the effect of sCD40L on platelets, resulting in decreased thrombotic and inflammatory effects of sCD40L. Interestingly, others have shown that concentrations of GP IIb/IIIa antagonists that give less than robust inhibition of platelet aggregation actually potentate the release of sCD40L (14). Orally available GP IIb/IIIa antagonists typically are administered at suboptimal doses to prevent nuisance bleeding. It remains to be determined whether these suboptimal doses cause the increased generation of sCD40L, contributing to the observed trend of a negative benefit in clinical trials with these drugs (17). Aspirin, inhibiting COX-1, and clopidogrel, a commonly used anti-platelet drug that inhibits the P2Y12 ADP receptor, also block sCD40L release from platelets—but only in response to collagen and adenosine diphosphate (14,18). Whether this will also be reflected by a lessening of the anti-thrombotic and anti-inflammatory effects of these medications compared with GP IIb/IIIa antagonist remains to be determined. The cleavage and release of sCD40L may involve an intermediate step, such as activation of the CD40L protease, or modification of the substrate, CD40L (3,12). Precedent exists for the interaction of $beta_3$-integrins with MMPs, the class of proteinases implicated in the shedding of ligands in the tumor necrosis factor-alpha superfamily. The findings by Furman et al. (13) further support such a notion by demonstrating that the release of sCD40L from platelets is at least partly regulated by actin polymerization and an MMP-inhibitor-sensitive pathway. Future studies will clarify whether these mechanisms represent suitable targets for blocking sCD40L release.

The anuclear platelet is being rediscovered as an intriguing link between thrombosis and inflammation, and CD40L-CD40 interaction seems to play an important role in this process. This ligand has the unique property of promoting both inflammatory and thrombotic responses; activating endothelial cells, leukocytes, and platelets; and operating in a self-perpetuating feedback loop. However, several questions remain to be resolved. Thus, although several studies have demonstrated thrombotic and inflammatory effects of recombinant trimeric sCD40L, the effects of natural sCD40L have been questioned (15). Also, an important question is whether the concentrations of sCD40L can become high enough to be biologically relevant in the plaque even if this is not reflected in the concentration of sCD40L in the circulation (13). Moreover, the mechanisms of sCD40L release in platelets are far from clear and will have to be studied further. Furthermore, the ability of anti-platelet drugs—and their optimal dosage—to inhibit sCD40L release or sCD40L-CD40/sCD40L-GP IIb/IIIa interaction on platelets will have to be clarified. Also, some thromboembolic complications were reported in both $CD40L^{-/-}$ mice and humans receiving antibodies to CD40L, reflecting the possibility that inhibition of platelet CD40L might render platelet plugs unstable (12,19). On the other hand, the inhibition of CD40L effects could help to prevent thrombus formation, and the exact role of sCD40L in thrombus formation and stabilization and the clinical consequences of anti-CD40L therapy is, at present, not clear. All these issues will have to be further clarified in forthcoming studies that provide greater insight into platelet-dependent mechanisms involved in the regulation of vascular and inflammatory disease, possibly leading to new treatment modalities in these disorders.

Reprint requests and correspondence: Dr. Pål Aukrust, Section of Clinical Immunology and Infectious Diseases, Rikshospitalet, 0027 Oslo, Norway. E-mail: pal.aukrust@rikshospitalet.no.

REFERENCES

1. Ruggeri ZM. Platelets in atherothrombosis. Nat Med 2002;8:1227–34.
2. Aukrust P, Wæhre T, Damås JK, Gullestad L, Solum NO. Inflammatory role of platelets in acute coronary syndromes. Heart 2001;86:605–6.
3. Anand SX, Viles-Gonzalez JF, Badimon JJ, Cavusoglu E, Marmur JD. Membrane-associated CD40L and sCD40L in atherothrombotic disease. Thromb Haemost 2003;90:377–84.
4. Aukrust P, Müller F, Ueland T, et al. Enhanced levels of soluble and membrane-bound CD40 ligand in patients with unstable angina—possible reflection of T lymphocyte and platelet involvement in the pathogenesis of acute coronary syndromes. Circulation 1999;100:614–20.
5. Schonbeck U, Varo N, Libby P, Buring J, Ridker PM. Soluble CD40L and cardiovascular risk in women. Circulation 2001;104:2266–8.

6. Henn V, Slupsky JR, Grafe M, et al. CD40 ligand on activated platelets triggers an inflammatory reaction of endothelial cells. Nature 1998;391:591–4.
7. Lutgens E, Gorelik L, Kleutjens KB, et al. Requirement for CD154 in the progression of atherosclerosis. Nat Med 1999;5:1313–6.
8. Lutgens E, Cleutjens KB, Heeneman S, et al. Both early and delayed anti-CD40L antibody treatment induces a stable plaque phenotype. Proc Natl Acad Sci USA 2000;97:7464–9.
9. Andre P, Srinivasa Prasad KS, Denis CV, et al. CD40L stabilizes arterial thrombi by a β3 integrin-dependent mechanism. Nat Med 2002;8:247–52.
10. Srinivasa Prasad KS, Andre P, He M, et al. Soluble CD40 ligand induces b3 intergrin tyrosine phosphorylation and triggers platelet activation by outside-in signaling. Proc Natl Acad Sci USA 2003;100: 12367–71.
11. Inwald DP, McDowall A, Peters MJ, Callard RE, Klein NJ. CD40 is constitutively expressed on platelets and provides a novel mechanism for platelet activation. Circ Res 2003;92:1041–8.
12. Henn V, Steinbach S, Buchner K, et al. The inflammatory action of CD40 ligand (CD154) expressed on activated human platelets is temporally limited by coexpressed CD40. Blood 2001;98:1047–54.
13. Furman MI, Krueger LA, Linden MD, Barnard MR, Frelinger AL III, Michelson AD. Release of soluble CD40L from platelets is regulated by glycoprotein IIb/IIIa and actin polymerization. J Am Coll Cardiol 2004;43:2319–25.
14. Nannizzi-Alaimo L, Alves VL, Phillips DR. Inhibitory effects of glycoprotein IIb/IIIa antagonists and aspirin on the release of soluble CD40 ligand during platelet stimulation. Circulation 2003;107: 1123–8.
15. Topol EJ, Ferguson JJ, Weisman HF, et al. Long-term protection from myocardial ischemic events in a randomized trial of brief integrin beta3 blockade with percutaneous coronary intervention: EPIC Investigator Group. JAMA 1997;278:479–84.
16. May AE, Kalsch T, Massberg S, et al. Engagement of glycoprotein IIb/IIIa (α(IIb)β3) on platelets upregulates CD40L and triggers CD40L-dependent matrix degradation by endothelial cells. Circulation 2002;106:2111–7.
17. Bhatt DL, Topol EJ. Current role of platelet glycoprotein IIb/IIIa inhibitors in acute coronary syndromes. JAMA 2000;284:1549–58.
18. Hermann A, Rauch BH, Braun M, et al. Platelet CD40 ligand (CD40L)-subcellular localization, regulation of expression, and inhibition by clopidogrel. Platelets 2001;12:74–82.
19. Kawai T, Andrews D, Colvin RB, et al. Thromboembolic complications after treatment with monoclonal antibody against CD40 ligand. Nat Med 2000;6:114.